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First occurrence of *Nigrospora lacticolonia* Mei Wang & L. Cai (Xylariales, Ascomycota) in the Neotropical Region

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Abstract

During a study on fungal endophyte diversity, *Nigrospora lacticolonia* Mei Wang & L. Cai was isolated from healthy leaves of *Guarea macrophylla*, a shade tree in the Cocoa agroecosystem (cabruca) in Brazil. We confirmed the identity of the specimens using morphological data and a phylogenetic reconstruction based on molecular markers (internal transcribed spacer region (ITS), β-tubulin (TUB2), and translation elongation factor 1-α (TEF) sequences). The specimen presented black globose or slightly ellipsoidal conidia, and the conidiophores were reduced to conidiogenous cells. This is the first report of *N. lacticolonia* in the Neotropical Region.

Keywords

Atlantic Forest, Cocoa crop, endophytic fungi, *Guarea macrophylla*, Sordariomycetes

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Introduction

Nigrospora was described by Zimmermann (1902) to accommodate *N. panici* Zimm. The genus belongs to the family Apiosporaceae, and it comprises 35 species according MycoBank (data from 2021). *Nigrospora* is characterized as presenting micronematous or semi-macronematous conidiophores, which are branched, flexuous, hyaline to brown, smooth, and usually reduced to conidiogenous cells; conidiogenous cells are monoblastic, dolliform, ampulliform, subcylindrical to clavate, and hyaline. Conidia are solitary, spherical or broadly ellipsoidal or pyriform, compressed dorsiventrally, black, shiny, smooth, and aseptate (Rathod et al. 2014; Donayre and Dalisay 2016; Wang et al. 2017; Raza et al. 2019).

Nigrospora species have been found to be pathogenic (Dutta et al. 2014a; Kwon et al. 2016; Kee et al. 2019), saprobic (Brown et al. 1998), and endophytic (Pawle and Singh 2014; Thanabalasingam et al. 2015). Endophytic fungi live inside plant tissues and do not harm their host (Azevedo and Araújo 2007); they produce secondary metabolites that promote plant growth (Khan et al. 2015), protect against disease (Dutta et al. 2014b), and

provide tolerance to ambient stresses (Jia et al. 2016). Endophytic species of *Nigrospora* have been reported in several hosts, such as *Cocos nucifera* (Oliveira et al. 2021), *Emblica officinalis* (Rathod et al. 2014), and *Artemisia* spp. (Cosoveanu 2016).

Wang et al. (2017) described *Nigrospora lacticolonia* for the first time in leaves of *Camellia sinensis* and *Musa paradisiaca* in China. It has been reported as a pathogenic fungus causing reddish brown spots on the stem of dragon fruit in Malaysia (Kee et al. 2019), and it is associated with leaf spots of sugarcane in China (Raza et al. 2019). Recently, *N. lacticolonia* has been newly reported as a leaf pathogen in the date palm in Oman, causing dark brown to black spots (Al-Nadabi et al. 2020). Our study represents the first report of *N. lacticolonia* in the Neotropical Region, where it was found living as an endophyte in healthy leaves of *Guarea macrophylla*, which is planted as a shade tree in the agroecosystems of Cocoa (*Theobroma cacao* L.) cultivation in Brazil.

Methods

Isolation and purification. *Nigrospora lacticolonia* (Table 1) was obtained from healthy leaves of *G. macrophylla* in a Cocoa agroecosystem (14°47′42″S, 039°10′20″W), Bahia, Brazil (Fig. 1). Following the methodology proposed by Araújo et al. (2002), the leaves were washed in running water and neutral detergent and later fragmented into leaf discs (6 mm in diameter) and subjected to surface disinfection with 70% alcohol (1 min), 3% sodium hypochlorite (NaOCl) (2 min and 30 s), again with 70% alcohol (30 s), and then washed with sterile distilled water. The discs were transferred to Petri dishes containing Malto-Dextrose Agar (MEA) plus chloramphenicol (50 mg/L) and incubated at room temperature (28 ± 2 °C).

Morphological study. The endophytic fungus was cultivated on potato dextrose agar (PDA) (Gams et al. 1998) and synthetic nutrient-poor agar (SNA) (Nirenberg 1976) for six days (28 ± 2 °C), and microscopic characteristics

Table 1. Isolate numbers, host, locality, lifestyle, and GenBank accession numbers of *Nigrospora lacticolonia* samples analysed.

Isolate number	Host	Locality	Lifestyle	GenBank accession numbers		
				ITS	TUB2	TEF1-α
LC 3324 = CGMCC3.18123	*Camellia sinensis*	China	—	KX985978	KY019458	KY019291
URM 8360	*Guarea macrophylla*	Brazil	Endophytic	MW838186	MW901250	MW886097
PC KS6B1 A R2	*Hylocereus polyrhizus*	Malaysia	Pathogenic	MK408578	MK408562	MK408567
LC 7009	*Musa paradisiaca*	China	—	KX986087	KY019594	KY019454
SQUCC 2269	*Phoenix dactylifera*	Oman	Pathogenic	MN173587	MN205976	MN205981
LC 12061	*Saccharum officinarum*	China	Pathogenic	MN215785	MN329949	MN264024

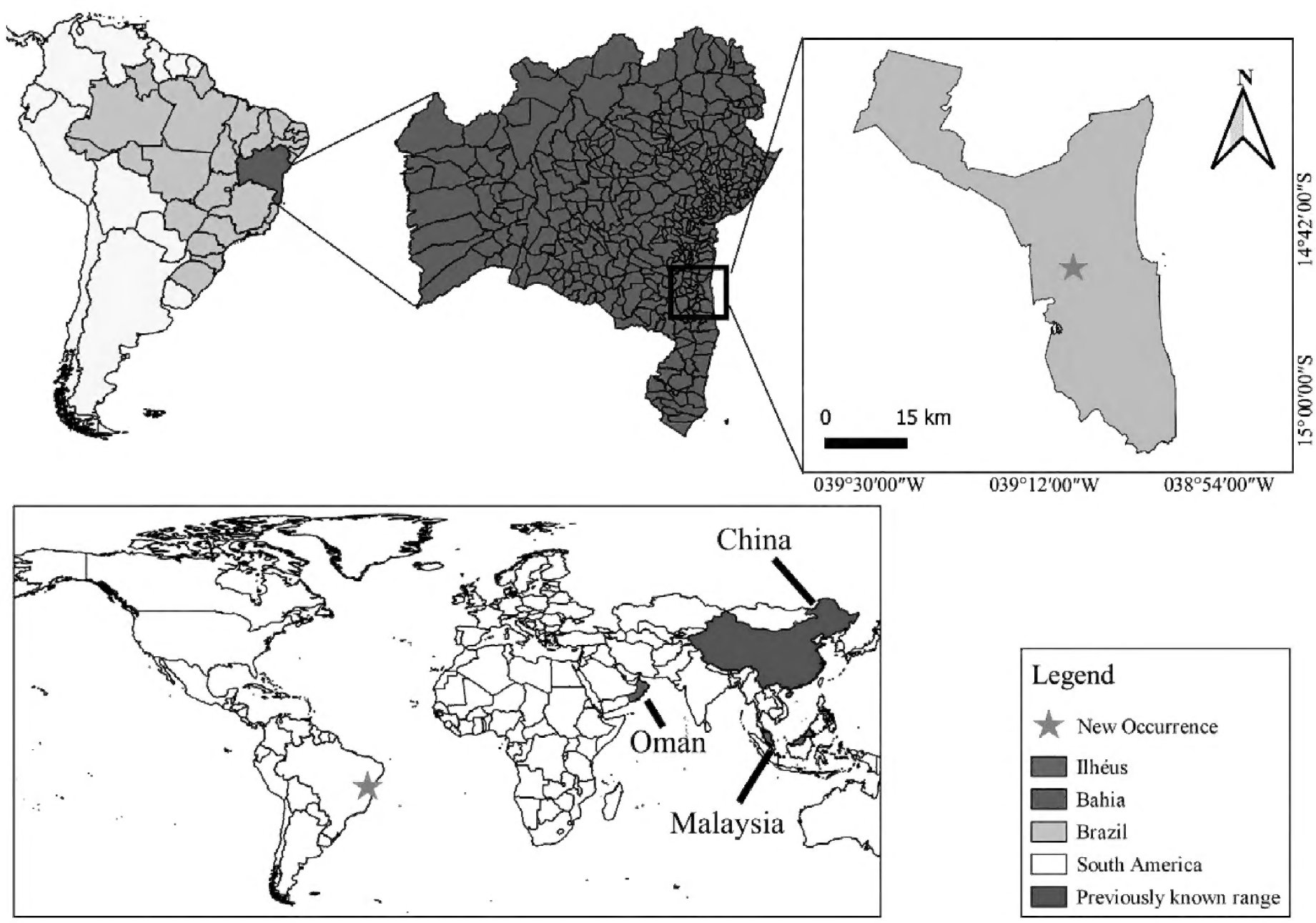

Figure 1. New occurrence of *Nigrospora lacticolonia* URM 8360 and previous distribution.

analysis was performed in SNA, to check the size of at least 20 conidia, 10 conidiogenous cells, and 10 hyphae. The isolate used in this study was maintained in the URM culture collection (Micoteca URM Profa. Maria Auxiliadora de Queiroz Cavalcanti) at the Universidade Federal de Pernambuco, Recife, Brazil.

Molecular analysis. For DNA extraction, fungal biomass was cultivated in a Petri dish containing PDA for seven days at 28 °C. The protocol used followed the methodology described by Oliveira et al. (2016). The primers ITS1/ITS4 (White et al. 1990) were used to amplify the rDNA ITS region using the parameters described by Oliveira et al. (2014). The primers Bt2a/Bt2b (Glass and Donaldson 1995), EF1-728F (Carbone and Kohn 1999) and EF-2 (Odonnell et al. 1998) were also used to amplify the TUB2 and TEF1-α genes, respectively. The sample was purified using NucleoSAP® (Cellco Biotec, São Carlos, SP, Brazil), according to the manufacturers' recommendations using the GeneAmp® PCR System 9700. Subsequently, the sample were sequenced using the same PCR primers sets by Multi-user DNA Sequencing Platform at the Biosciences Center, Universidade Federal de Pernambuco, Recife, Brazil. The consensus sequences were computed and visually inspected using the Staden software package (Staden et al. 1998).

Phylogenetic analysis. Phylogeny was reconstructed based on the combination of the rDNA ITS region, TUB2, and TEF1-α genes. The sequence of *N. lacticolonia* was aligned with others retrieved from GenBank using MEGA v. 5.05 (Tamura et al. 2007). Prior to phylogenetic analyses, the optimal model of nucleotide substitution (TRN + G) was estimated using Topali v. 2.5 (Milne et al. 2004). A maximum likelihood (1.000 bootstraps) analysis was performed using the PhyMl (Guindon and Gascuel 2003), launched from Topali v. 2.5 (Milne et al. 2004). *Arthrinium malaysianum* CBS 102053 was including as the out-group.

Results

Nigrospora lacticolonia **Mei Wang & L. Cai, 2017**; Persoonia 39: 131.
Figure 2A–E

Material examined. BRAZIL – Bahia • Ilhéus, Universidade Estadual de Santa Cruz; 14°47′42″S, 039°10′20″W; 05.VII.2019; Deyse Viana dos Santos leg.; as

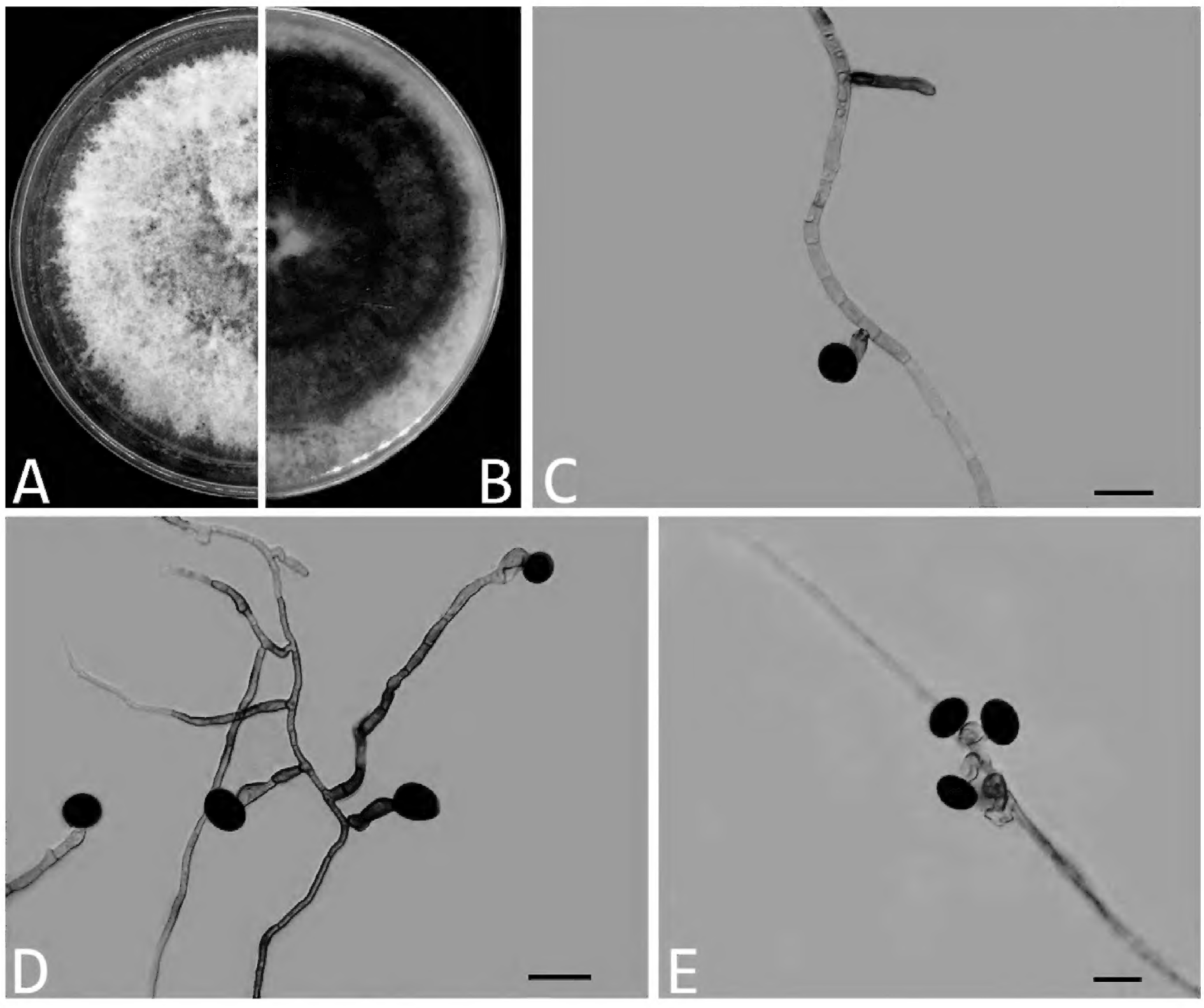

Figure 2. *Nigrospora lacticolonia*. **A, B.** Top and bottom view of the colony, six days after inoculation on PDA. **C, D, E.** Conidiogenous cells and conidia. Scale bars = 10 µm.

endophytic fungi in healthy leaves of *Guarea macrophylla*; URM 8360.

Identification. After 6 days at 28 ± 2 °C on SNA, hyphae smooth, hyaline to light brown, branched, septate, 2–4 µm in diameter. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, pale brown, globose to clavate to dolliform, 7.5–12.5 × 5–10 µm. Conidia were black, shiny, smooth, aseptate, globose, or slightly ellipsoidal 12.5–15.0 × 10–15 µm.

Culture characteristics. On PDA, colonies floccose, surface white, reverse light brown, reaching 9 cm in diameter after 6 days at 28 ± 2 °C. On SNA, surface and reverse mycelium black, colonies flat. The phylogenetic tree (Fig. 3) shows that the URM 8360 sequence formed a clade together with *N. lacticolonia* sequences, including the holotype (CGMCC3.18123).

Discussion

Based on morphological characteristics and phylogenetic analysis, *N. lacticolonia* has been identified and is reported for the first time in the Neotropical Region from a Cocoa agroecosystem in the Atlantic Forest domain. Wang et al. (2017) confirmed *Nigrospora* as a monophyletic genus and described *N. lacticolonia* as present on the leaves of *Camellia sinensis* and *Musa paradisiaca* in China. The morphological characteristics of the specimen *N. lacticolonia* URM 8360 were similar to those described by Wang et al. (2017): hypha size (1.5–4.0 µm in diameter (LC 3324) vs. 2.0–4.0 µm in diameter), conidia size (13.5–17.5 × 10.5–13.5 µm (LC 3324) vs. 12.5–15.0 × 10.0–15.0 µm), and conidiogenous cell size (6.5–11.5 × 5.5–9.0 µm (LC 3324) vs. 7.5–12.5 × 5.0–10.0 µm). According to our BLASTn analyses, the ITS rDNA, TUB2, and TEF1-α sequences obtained from our

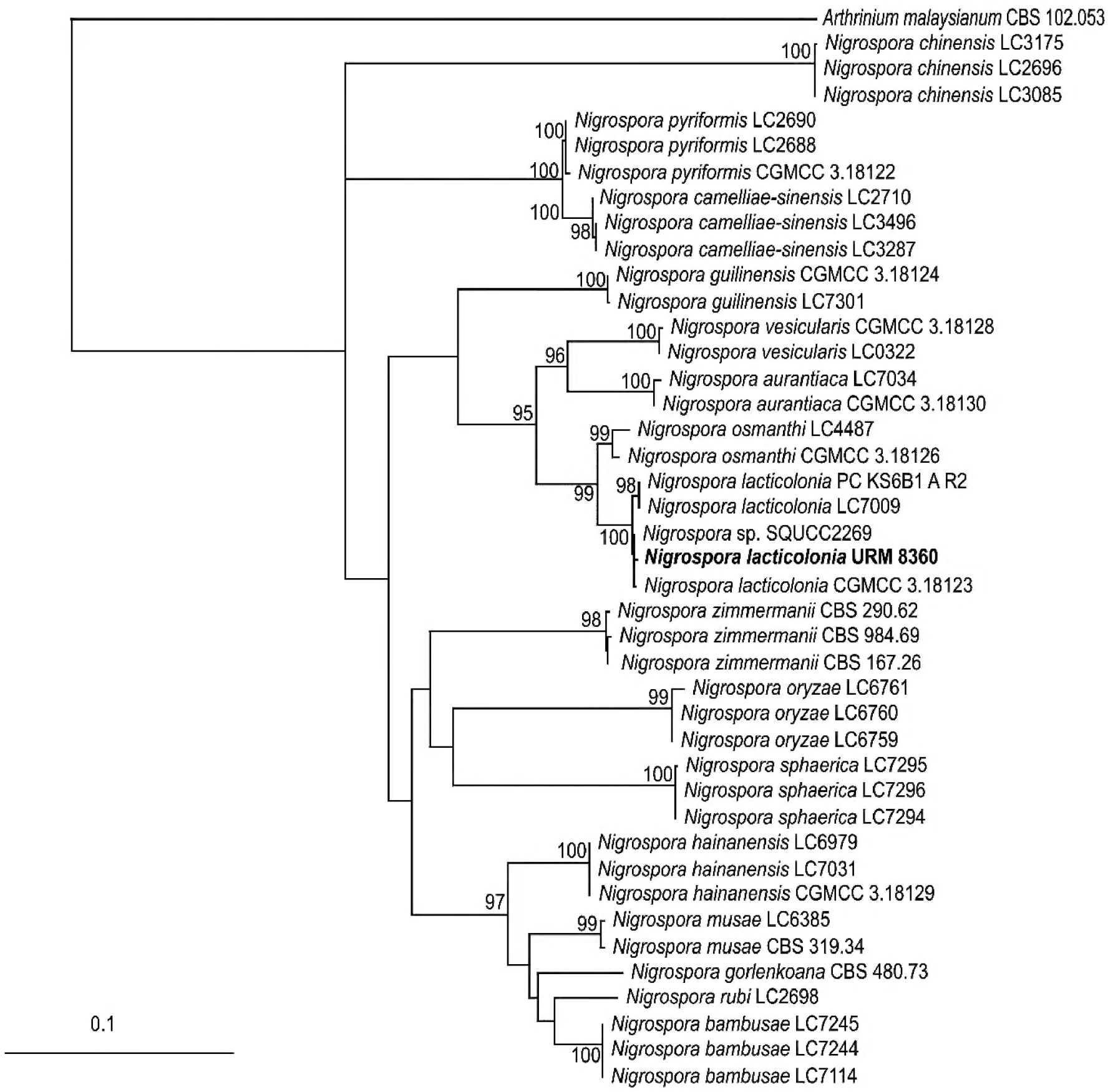

Figure 3. Phylogenetic tree of *Nigrospora lacticolonia* and related species, constructed using combined rDNA ITS region, TUB2, and TEF1-α genes. *Arthrinium malaysianum* CBS 102.053 was used as outgroup. Support values were obtained from maximum likelihood analysis. The sequence obtained in this study is annotated in bold.

specimens were 99.31%, 99.67%, and 100% identical to the KX986087 (LC 7009), K408564 (PC KS6B1 B R2), and KY019291 (CGMCC3.18123) sequences, respectively, of *N. lacticolonia* from GenBank.

There are reports of *N. lacticolonia* as a pathogenic fungus on leaves of Date Palm (*Phoenix dactylifera* L.) in Oman (Al-Nadabi et al. 2020), causing reddish brown spots on the stems of *Hylocereus polyrhizus* (Weber) Britton & Rose in Malaysia (Kee et al. 2019), and disease symptoms in leaves of sugarcane (*Saccharum officinarum* L.) in China (Raza et al. 2019). However, in this study, *N. lacticolonia* is reported as endophyte on *G. macrophylla* (family Meliaceae), a native tree of the Atlantic Forest (Flores 2020). It is used as a shade tree in the Cocoa crop system (Sambuichi 2003).

We report here the first record of *N. lacticolonia* in the Neotropics and as endophyte in a new host plant species. Our increases the knowledge of the geographic distribution and lifestyle of this species and emphasizes the importance of further studies on fungal endophytes in agroecosystems worldwide.

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